

Appl. No. 09/373,403
Amendment dated March 3, 2006
Reply to Advisory Action of October 24, 2005

Amendments to the Claims:

This listing of claims will replace all prior versions and listing of claims in the application.

Listing of Claims:

1-29. (Cancelled)

30. (Currently Amended) A method of preparing a multispecific antibody comprising a first polypeptide and at least one additional polypeptide, wherein

(a) the first polypeptide comprises a multimerization domain forming an interface positioned to interact with an interface of a multimerization domain of the additional polypeptide,

(b) the first and additional polypeptides each comprise a binding domain, the binding domain comprising a heavy chain and a light chain, wherein each binding domain binds to a different antigen, wherein the light chains of the first and additional polypeptides each have three CDR regions, and have at least 98% sequence identity to one another and only differ from one another at amino acid positions outside of the CDR regions, the method comprising the steps of:

(i) culturing a host cell comprising a nucleic acid encoding the first polypeptide and a nucleic acid encoding at least one additional polypeptide, wherein the culturing is such that the nucleic acids are expressed; and

(ii) recovering the multispecific antibody from the host cell culture.

31. (Previously Presented) The method of claim 30, wherein the nucleic acid encoding the first polypeptide or the nucleic acid encoding the additional polypeptide, or both, has been altered from an original nucleic acid to encode the interface or a portion thereof.

32. (Previously Presented) The method of claim 31 wherein the multimerization domains of one of the first or additional polypeptides, or both, are altered to comprise a free thiol-containing residue which is positioned to interact with a free thiol-containing residue of the interface of the

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other of the first or additional polypeptide such that a disulfide bond is formed between the first and additional polypeptides, wherein the nucleic acid encoding the first polypeptide has been altered from the original nucleic acid to encode the free thiol-containing residue or the nucleic acid encoding the additional polypeptide has been altered from the original nucleic acid to encode the free thiol-containing residue, or both.

33. (Previously Presented) The method of claim 30 wherein the multimerization domains of the first and additional polypeptides comprise a protuberance-into-cavity interaction, wherein the method further comprises:

generating a protuberance by altering the original nucleic acid encoding the first polypeptide to encode the first polypeptide with an import residue having a larger side chain volume than the original residue, and

generating a cavity by altering a portion of the original nucleic acid encoding the additional polypeptide to encode the additional polypeptide with an import residue having a smaller side chain volume than the original residue.

34. (Previously Presented) The method of claim 33, wherein the steps of generating a protuberance or generating a cavity, or both, occurs by phage display selection.

35. (Previously Presented) The method of claim 33 wherein the import residue having a larger side chain volume than the original residue is selected from the group consisting of arginine (R), phenylalanine (F), tyrosine (Y), tryptophan (W), isoleucine (I) and leucine (L).

36. (Previously Presented) The method of claim 33 wherein the import residue having a smaller side chain volume than the original residue is selected from the group consisting of glycine (G), alanine (A), serine (S), threonine (T), and valine (V), and wherein the import residue is not cysteine (C).

37. (Previously Presented) The method of claim 30 wherein the first and additional polypeptide each comprise an antibody constant domain.

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38. (Currently Amended) The method of claims 37 wherein the first and additional polypeptide each comprise an antibody constant domain selected from the group consisting of a C₁₁₃ domain and an IgG.
39. (Previously Presented) The method of claim 30 wherein the antibody is a multispecific immunoadhesin.
40. (Previously Presented) The method of claim 30 wherein step (i) is preceded by a step of introducing the nucleic acid encoding the first and additional polypeptide into the host cell.
41. (Previously Presented) An isolated host cell comprising the nucleic acids encoding the multispecific antibody of claim 30.
42. (Previously Presented) The host cell of claim 41 wherein the host cell is a mammalian cell.
43. (Currently Amended) A method of preparing a multispecific antibody comprising:
 - (a) selecting a first nucleic acid encoding a first polypeptide comprising a heavy chain from an antibody specific for a first antigen and an interface comprising an altered amino acid residue in an interface of the first polypeptide, and selecting at least one additional nucleic acid encoding the at least one additional polypeptide comprising a heavy chain from an antibody specific for a second antigen and an interface, wherein the interface has so that an amino acid residue on the additional polypeptide that specifically interacts with the altered amino acid residue in the interface on the first polypeptide, thereby generating a stable interaction between the first and said additional polypeptides;
 - (b) selecting a light chain encoding nucleic acid sequence, wherein the light chain associates with a binding region of each first and additional polypeptide of the multispecific antibody, wherein the binding region comprises a heavy chain and the light chain and wherein each binding region binds to a different antigen;

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(c) introducing into a host cell the first and additional nucleic acids and the light chain-encoding nucleic acid, and culturing the cell so that expression of the first and additional nucleic acids and the light chain-encoding nucleic acid occurs to form a multispecific antibody;

(d) recovering the multispecific antibody from the cell culture.

44. (Cancelled)

45. (Previously Presented) The method of claim 43, wherein the altering comprises generating a protuberance-into-cavity interaction at the interface between the first and additional polypeptides.

46. (Previously Presented) The method of claim 43, wherein the altering comprises importing a free thiol-containing residue into the first or additional polypeptide or both, such that the free thiol-containing residues interact to form a disulfide bond between the first and additional polypeptides.

47. (Previously Presented) The method of claim 43 wherein the first and additional polypeptide each comprise an antibody constant domain.

48. (Previously Presented) The method of claim 47 wherein the antibody constant domain is a C_H3 domain.

49. (Previously Presented) The method of claim 48 wherein the antibody constant domain is from a human IgG.

50. (Currently Amended) A method of preparing a multispecific antibody comprising a first polypeptide and at least one additional polypeptide, wherein

(a) the first polypeptide comprises a multimerization domain forming an interface positioned to interact with an interface of a multimerization domain of the additional polypeptide,

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(b) the first and additional polypeptides each comprise a binding domain, the binding domain comprising a heavy chain and a common light chain, wherein the common light chain of the first and additional polypeptides has at least 98% sequence identity to each a variable domain of a light chain of a first antibody and/or at least one additional antibody and only differs from each of the light chains of the first and/or at least one additional antibody at amino acid positions outside of the CDR regions, and wherein the first and at least one additional antibody bind to different antigens, the method comprising the steps of:

(i) culturing a host cell comprising nucleic acid encoding the first polypeptide and additional polypeptides, and the common light chain, wherein the culturing is such that the nucleic acid is expressed; and

(ii) recovering the multispecific antibody from the host cell culture.

51. (Currently Amended) The method of claim 50, wherein each [[the]] common light chains have has 100% identity to each variable domain of a light chain of a first antibody and at least one additional antibody.

52. (Cancelled)

53. (Previously Presented) The method of claim 50, wherein each of the first and second multimerization domains comprise a C_H3 domain of an antibody constant domain.

54. (Previously Presented) The method of claim 53, wherein the first multimerization domain has a protuberance and the second multimerization domain has a cavity and the first and second multimerization domains dimerize by the fitting of the protuberance into the cavity.

55. (Previously Presented) The method of claim 54, wherein the multimerization domain also comprises a non-naturally occurring disulfide bond.